Allergology International (1997) **46**: 117–124

Original Article

Scratching behavior in mice associated with IgE-mediated allergic cutaneous reaction and its pharmacological characterization

Keiichi Musoh, Nobuaki Nakamura, Toshimi Sakurai, Naoki Inagaki and Hiroichi Nagai

Department of Pharmacology, Gifu Pharmaceutical University, Gifu, Japan

ABSTRACT

Scratching behavior observed after epicutaneous challenge with the antigen 2,4-dinitrofluorobenzene (DNFB) in the ear of BALB/c mice passively sensitized with anti-dinitrophenol (DNP). Immunoglobulin (Ig) E was characterized pharmacologically and compared with that caused by compound 48/80. Although DNFB application itself caused scratching behavior in non-sensitized mice, the number of scratchings apparently increased in sensitized mice from 60 min after antigen application in comparison with non-sensitized control mice. Prednisolone, cyproheptadine, dibucaine and naloxone significantly inhibited the DNFB-induced scratching behavior, whereas the histamine H_1-receptor antagonists HSR-609, cetirizine and terfenadine only showed a tendency to inhibit scratching. Injection of 48/80 into the rostral part of the back also caused scratching. The first scratching was observed within 10 min after injection and lasted intermittently for 30 min. The 48/80-induced scratching was markedly inhibited by cyproheptadine, dibucaine and naloxone, but not by prednisolone and the histamine H_1-receptor antagonists. Ear edema caused by DNFB application in sensitized mice was markedly inhibited by prednisolone, HSR-609, cetirizine, terfenadine and cyproheptadine, whereas dibucaine and naloxone failed to affect ear edema. These results indicate that scratching behavior could be induced in mice in association with an IgE-mediated allergic cutaneous reaction and that the reaction is pharmacologically similar, but not identical, to that caused by 48/80. Although histamine is considered to participate in the formation of ear edema, it may not play an important role in the generation of scratching.

Key words: antihistamine, compound 48/80, cyproheptadine, dinitrofluorobenzene, IgE-mediated allergic cutaneous reaction, prednisolone, scratching behavior.

INTRODUCTION

The itch is a sensation associated with a strong desire to scratch. In many diseases, such as allergic dermatitis, chronic renal failure, hepatic cholestasis and diabetes mellitus, pruritus is one of the most important symptoms.[1–4] Particularly in skin diseases, pruritus with an extremely unpleasant sensation is the most significant problem, but the mechanisms causing itching remain to be elucidated. It is necessary, therefore, to clarify the physiological and pathological mechanisms of pruritus. At present, however, no established animal model is available, because of the difficulties in behavioral animal experiments, to investigate pruritus. Clinically, scratch is used as an objective measure of itch. In 1995, Kuraishi *et al.*[5] reported that scratching in mice caused rostral back injection of the pruritogenic agents compound 48/80 (48/80) and substance P may be due to itch, but not pain. They also indicated that histamine and algesiogenic agents, capsaicin and formalin, were without significant effects.

In contrast, application of 2,4-dinitrofluorobenzene (DNFB), a typical contact sensitizer, on the ear of mice sensitized with anti-dinitrophenol (DNP) IgE causes

Correspondence: Dr Hiroichi Nagai, Department of Pharmacology, Gifu Pharmaceutical University, 5–6-1 Mitahorahigashi, Gifu 502, Japan. Email: <QYJ05731@niftyserve.or.jp>

Received 12 September 1996. Accepted for publication 21 February 1997.

biphasic ear edema.[6–9] The immediate phase edema peaked 1 h after DNFB application, is transient and is considered to be generated by mast cell mediators, such as histamine and serotonin.[9] The late phase edema is long-lasting and shows a peak 24 h after antigen application. Pro-inflammatory cytokines, such as tumor necrosis factor-α and interleukin-1β, are suggested to be involved in the late-phase edema.[8,10]

In the present study, the scratching behavior caused by an allergic cutaneous reaction in mice was investigated according to the method reported by Kuraishi *et al.*[5] and attempts were made to pharmacologically characterize the scratching behavior.

METHODS

Animals

Female BALB/c mice, 8–9 weeks old, purchased from Japan SLC (Hamamatsu, Japan) were used throughout.

Antigen

2,4-Dinitrofluorobenzene, purchased from Nacalai Tesque (Kyoto, Japan), was used as an antigen; it was dissolved in a mixture of acetone and olive oil (3:1).

Monoclonal IgE

Mouse monoclonal IgE against DNP residue was prepared by culturing a cell line, EC1, as previously reported.[8] The culture supernatant of EC1 was stored at –80°C and was used as a source of IgE. The maximum dilution of the IgE preparation to give a positive cutaneous anaphylaxis in Wistar rats challenged with DNP-conjugated bovine serum albumin was 1:1024. The IgE content of the preparation, estimated by enzyme-linked immunosorbent assay, was 1415 ng/mL.

Drugs

3-[4-(8-Fluoro-5,11-dihydro [1] benzoxepino [4,3-*b*] pyridin-11-ylidene) piperidino] propionic acid (HSR-609), a novel amphoteric antiallergic agent with a potent and long-lasting histamine H_1-receptor antagonistic property,[11] and cetirizine dihydrochloride[12,13] were synthesized by Hokuriku Seiyaku Co. Ltd (Fukui, Japan). Terfenadine[14] was purchased from Sigma Chemical Co. (St Louis, MO, USA). These agents were suspended in 5% arabic gum solution. Naloxone hydrochloride (Sigma Chemical Co.), cyproheptadine hydrochloride (Nacalai Tesque), dibucaine hydrochloride (Nacalai Tesque) and compound 48/80 (Sigma Chemical Co.) were dissolved in physiological saline. Prednisolone acetate (Shionogi Pharmaceutical Co. Ltd, Osaka, Japan) was also suspended in physiological saline. HSR-609, cetirizine, terfenadine and cyproheptadine were administered orally to mice 1 h prior to the elicitation of any reaction. Naloxone was given intravenously 1 h before the reaction. Prednisolone was injected intraperitoneally 2 h before the reaction. Dibucaine was given to mice subcutaneously at the time of stimulation.

DNFB-induced scratching behavior

2,4-Dinitrofluorobenzene treatment in sensitized mice was performed as reported previously[8–10] with a slight modification. Briefly, mice received an intravenous injection of 1.0 mL monoclonal IgE preparation. Twenty-four hours after passive sensitization, DNFB acetone–olive oil solution was applied to both sides of the ears. Although we have observed mouse biphasic cutaneous reactions after applying 25 μL of a 0.15% DNFB solution, a high incidence of scratching was observed even in non-sensitized mice under such experimental conditions. We therefore painted 5 μL of a 0.75% DNFB solution on both sides of the ear to reduce non-specific scratching when examining scratching behavior. Immediately after antigen application, mice were put into acrylic cages (30×30×12 cm) and their behavior was observed. Instances of scratching of the ear lobes by the hind paws were counted. The mice showed several scratchings for approximately 1 s and a series of these behaviors was counted as one incident of scratching according to the method described by Kuraishi *et al.*[5]

48/80-induced scratching behavior

48/80 solution in a volume of 100 μL was injected subcutaneously into the rostral part of the back of mice.[5] Immediately after injection of 48/80, mice were put into acrylic cages and scratching behaviors were counted as mentioned above.

DNFB-induced edematous reaction

An IgE-dependent cutaneous reaction was performed in the mouse ear as reported previously.[8–10] The ear edema caused by the application of DNFB was evaluated by

measuring the ear thickness by a micrometer (Peacock Upright Dial Gauge, Ozaki, Tokyo, Japan) before and 1 h after antigen challenge.

Statistics

Data are expressed as the mean±SEM. Statistical comparisons were made by parametric Duncan's multiple range test, using the computer software YUKMS (version 5.0; Kanagawa, Japan) and $P < 0.05$ was taken to indicate significance.

RESULTS

DNFB-induced scratching behavior in sensitized mice

Figure 1a shows the results of a time course study of scratching behavior for 120 min after DNFB or vehicle application to the ears of sensitized or intact mice. Scratching behavior was caused by DNFB application itself in intact mice in comparison with vehicle-treated controls. The number of scratchings, however, significantly increased from 60 to 120 min after application of DNFB in sensitized mice compared with DNFB-treated intact mice. The incidence of scratching behavior declined thereafter (data not shown). Based on these results, scratching behavior was observed for 120 min in the following experiments.

As shown in Fig. 1b, prednisolone at doses of 1 and 3 mg/kg significantly inhibited scratching for 120 min in a dose-related manner.

Results of administration of HSR-609, cetirizine and terfenadine are shown in Fig. 1c–e. These three antihistamines only showed a tendency to inhibit scratching. The degree of inhibition of scratching for 120 min by HSR-609 (1 mg/kg), cetirizine (0.1 mg/kg) and terfenadine (100 mg/kg) was 41.2, 31.0 and 29.9%, respectively. In contrast to the antihistamines, cyproheptadine, an antagonist against histamine H_1- and serotonin receptors, at a doses of 10 mg/kg significantly inhibited the 120 min scratching counts (Fig. 1f).

Results of administration of the local anesthetic dibucaine are shown in Fig. 1g. Dibucaine, at a dose of 300 μg/site, significantly inhibited the scratching counts for 120 min, but did not inhibit scratching at a dose of 30 μg/site. Naloxone, an opiate receptor antagonist, at a dose of 3 mg/kg also significantly inhibited scratching counts for 120 min (Fig. 1h).

48/80-induced scratching in mice

When BALB/c mice received an injection of 48/80 at doses of 10 and 100 μg into the rostral part of the back, they exhibited scratching of or around the injected site by the hind paws. The first scratching behavior appeared within 10 min after injection and lasted intermittently for 30 min. 48/80 at 100 μg was effective in causing scratching, but was less effective at 10 μg. A dose of 1 μg 48/80 failed to induce scratching (Fig. 2a). The incidence of scratching caused by 100 μg 48/80 peaked between 10 and 15 min. As we had confirmed in preliminary experiments that the incidence of 48/80-induced scratching was low later than 45 min after its injection (data not shown), mouse behavior was only observed for 45 min after injection of 48/80 in the present experiments. In the following experiments, scratching behavior was caused by injecting 100 μg 48/80.

Results of administration of prednisolone are shown in Fig. 2b. Prednisolone failed to affect 48/80-induced scratching behavior in mice.

As shown in Fig. 2c–e, HSR-609, cetirizine and terfenadine did not affect 48/80-induced scratching in mice. In contrast to these antihistamines, cyproheptadine, at a dose of 10 mg/kg, significantly inhibited 48/80-induced scratching (Fig. 2f).

Results of dibucaine administration are shown in Fig. 2g. Dibucaine, at doses of 30 and 300 μg/site, significantly inhibited scratching for 45 min in a dose-related manner. As shown in Fig. 2h, naloxone at 10 mg/kg also significantly inhibited scratching behavior.

DNFB-induced edematous reaction in sensitized mice

Application of DNFB in the ear of sensitized mice caused a transient edematous reaction. The ear edema peaked 1 h after antigen challenge and declined thereafter (data not shown). Drug effects on ear edema, estimated at 1 h, are shown in Fig. 3.

Prednisolone at doses of 1 and 3 mg/kg potently inhibited ear edema. Three antihistamines, HSR-609 (at 0.1 and 1 mg/kg), cetirizine (at 0.1 and 1 mg/kg) and terfenadine (at 10 and 100 mg/kg), significantly inhibited ear edema. Cyproheptadine at a dose of 30 mg/kg also significantly inhibited the development of ear edema. However, dibucaine and naloxone did not affect the development of ear edema.

Discussion

In the present study we demonstrated that scratching by the hind paws could be induced by epicutaneous challenge with DNFB in mice passively sensitized with monoclonal IgE and we pharmacologically characterized this scratching behavior. Previously we have indicated that the IgE-dependent allergic cutaneous reaction is a suitable animal model for investigating the mechanism of allergic dermatitis, particularly the cutaneous late-phase

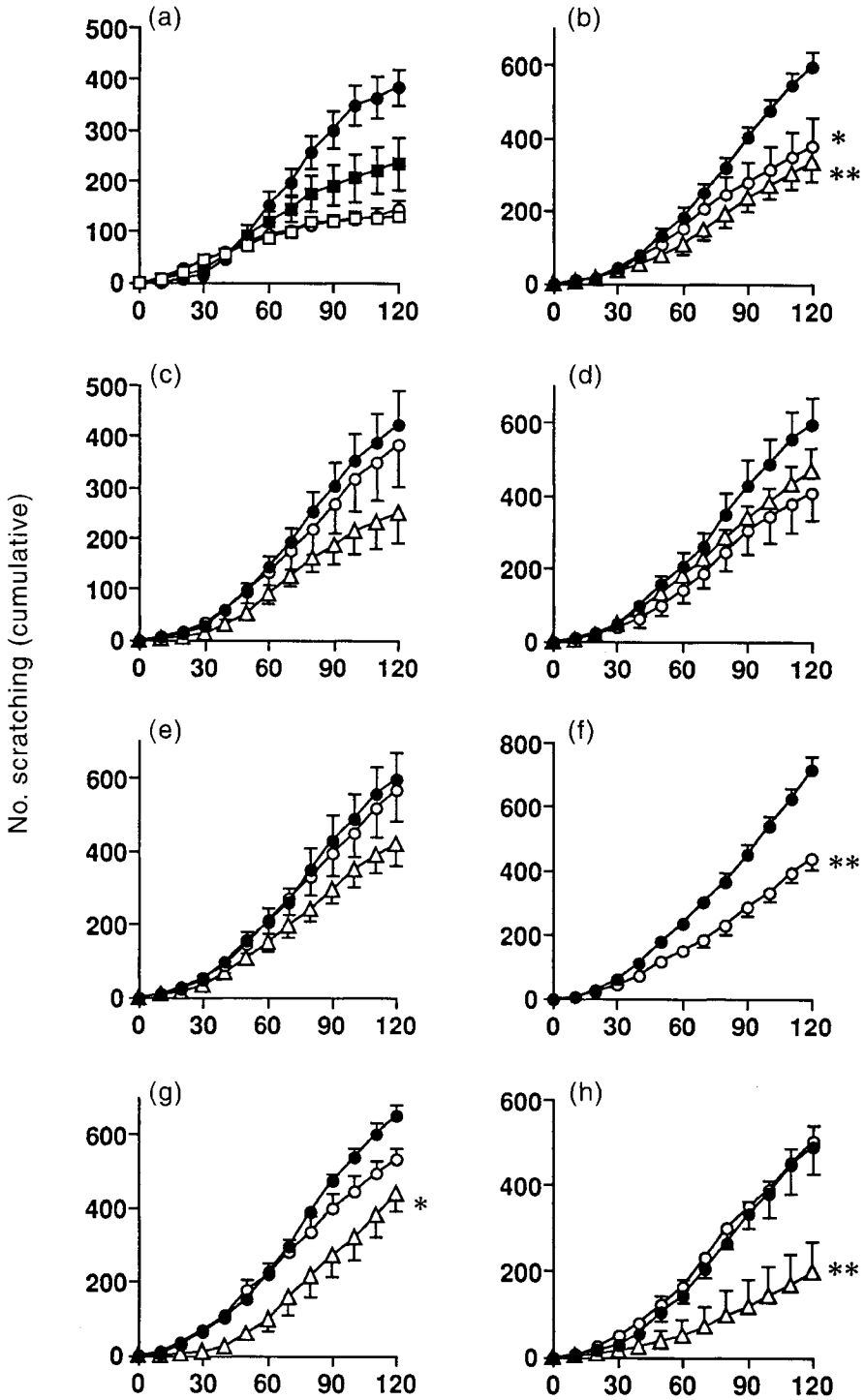

Fig. 1 2,4-Dinitrofluorobenzene (DNFB)-induced scratching in sensitized mice and the effects of various drugs on scratching. Mice were sensitized with anti-dinitrophenol IgE and were challenged with DNFB. Scratching behavior was counted for 120 min after DNFB application. (a) Basal experiment: (○), IgE+vehicle; (●), IgE+DNFB; (□), vehicle only; (■), DNFB only. (b) Prednisolone; (○), 1 mg/kg; (△), 3 mg/kg. (c) HSR-609: (○) 0.1 mg/kg; (△), 1 mg/kg. (d) Cetirizine: (○), 0.1 mg/kg; (△), 1 mg/kg. (e) Terfenadine: (○), 10 mg/kg; (△), 100 mg/kg. (f) Cyproheptadine: (○), 10 mg/kg. (g) Dibucaine: (○), 30 μg/site; (△), 300 μg/site. (h) Naloxone: (○), 1 mg/kg; (△), 3 mg/kg. Closed circles in b–h represent control. Each point and bar represent the mean±SEM of four to eight mice. Statistical evaluation was performed using total counts for 120 min (*$P<0.05$, **$P<0.01$).

reaction.[8–10] The present results indicate that this model may also be suitable for investigating pruritus caused by allergic dermatitis.

In 1995, Kuraishi *et al.*[5] reported that 48/80 induces scratching by the hind paws in ddY mice and that the scratching behavior is due to itch but not to pain. In human subjects, an intradermal or subcutaneous injection of 48/80 is also known to cause an itch sensation with a short duration.[15–17] Therefore, we examined scratching behavior due to an allergic

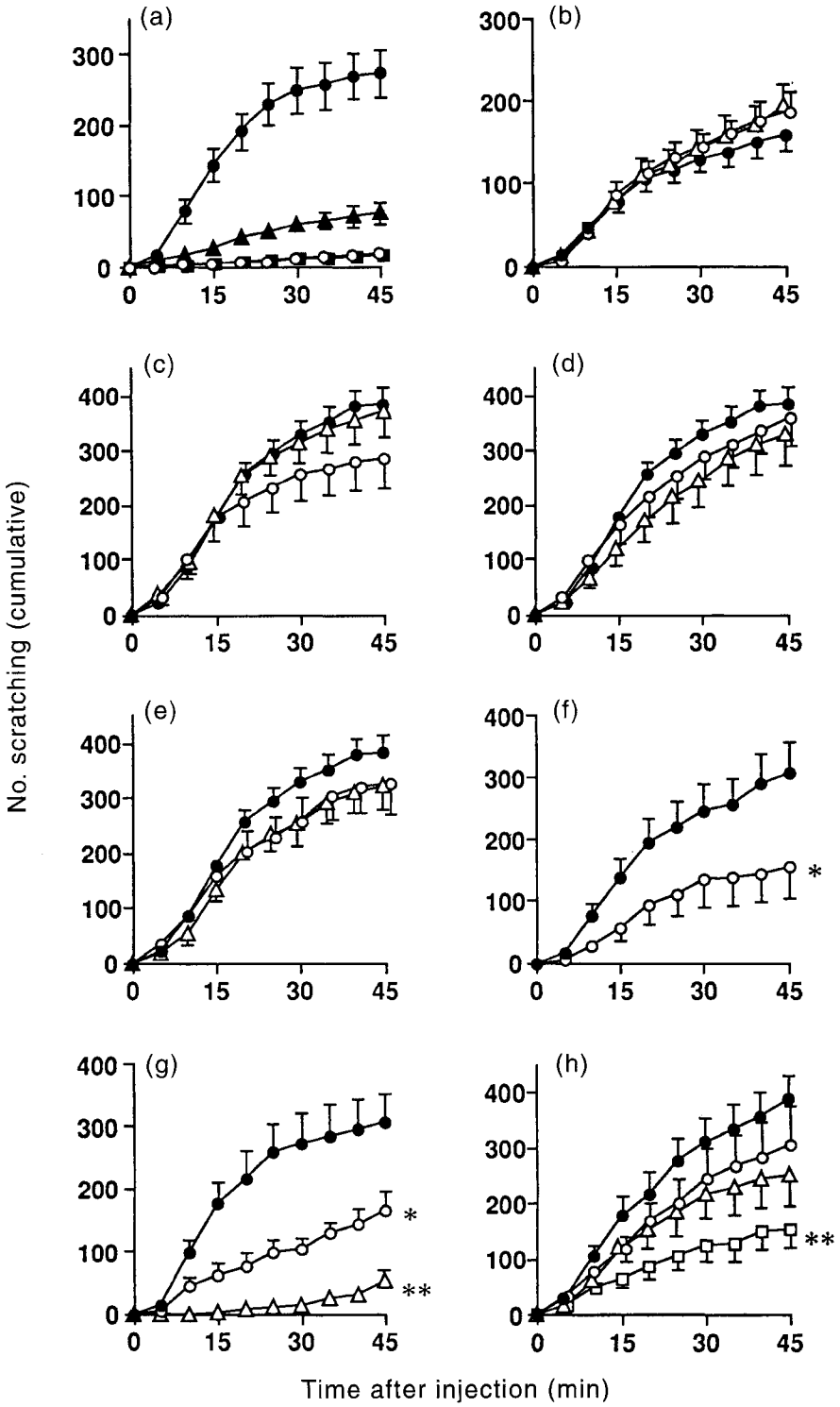

Fig. 2 48/80-induced scratching in mice and effects of various drugs on the scratching. Scratching behavior was counted for 45 min after a subcutaneous injection of 48/80. (a) Basal experiment: (○), saline, 100 μL/site; (■), 48/80, 1 μg/site; (▲), 48/80, 10 μg/site; (●), 48/80, 100 μg/site. (b) Prednisolone: (○), 1 mg/kg; (△), 3 mg/kg. (c) HSR-609: (○), 0.1 mg/kg; (△), 1 mg/kg. (d) Cetirizine: (○), 0.1 mg/kg; (△), 1 mg/kg. (e) Terfenadine: (○), 10 mg/kg; (△), 100 mg/kg. (f) Cyproheptadine: (○), 10 mg/kg. (g) Dibucaine: (○), 30 μg/site; (△), 300 μg/site. (h) Naloxone: (○), 1 mg/kg; (△), 3 mg/kg; (□), 10 mg/kg. Closed circles in b–h represent control. Each point and bar represent the mean±SEM of four to eight mice. Statistical evaluation was performed using total counts for 45 min (*$P<0.05$, **$P<0.01$).

reaction in comparison with 48/80-induced scratching.

Although the first scratching behavior caused by 48/80 was observed within 10 min after its injection, as reported by Kuraishi *et al.*,[5] the scratching behavior after DNFB application took some time to develop, which may be related to the period required for DNFB to penetrate through the skin. 2,4-Dinitrofluorobenzene application itself caused scratching behavior in non-sensitized mice.

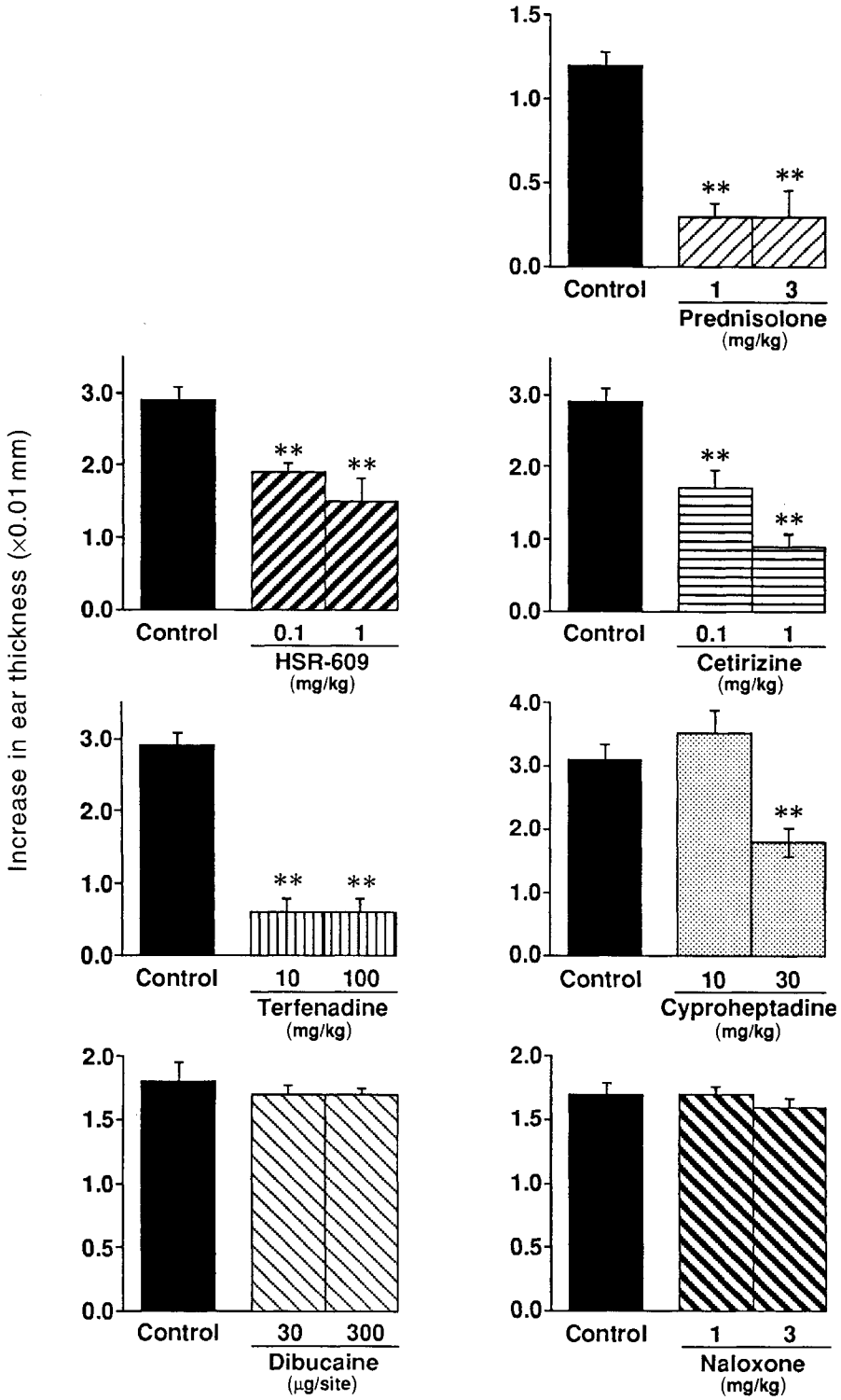

Fig. 3 Effects of various drugs on the 2,4-dinitrofluorobenzene (DNFB)-induced edematous reaction in sensitized mice. Mice were sensitized with anti-dinitrophenol IgE and were challenged with DNFB. The ear edema caused by the application of DNFB was evaluated by measuring ear thickness 1 h after antigen challenge. Each column and bar represent the mean±SEM of four to eight mice. $^{**}P<0.01$.

Vehicle application also caused scratching behavior in mice, although the incidence was lower than that induced by application of DNFB. In sensitized mice, however, the number of scratchings apparently increased from 60 min after DNFB application in comparison with non-sensitized control mice, indicating that the IgE-mediated allergic cutaneous reaction could be accompanied by scratching behavior in mice. 2,4-Dinitrofluorobenzene application to sensitized mice causes a biphasic ear edema with peak responses at 1 and 24 h after challenge.[6–9] The early phase edema was suppressed by mast cell stabilizers and antihistamines, and was not evoked in mast cell-deficient WBB6F1-W/W^{v} mice. The presence of degranulated mast cells has been confirmed in the early phase edema. In contrast, the late-phase edema was characterized by infiltration of neutrophils and macrophages. Therefore, DNFB-induced scratching behavior observed in the present study may be correlated to the mast cell-dependent immediate-phase edema, although the scratching behavior seemed to develop slightly slower than did the edema. Although the present data clearly indicate that a scratching behavior can be caused in mice in association with an IgE-mediated allergic cutaneous reaction, we could not distinguish the IgE-mediated scratching from non-specific scratching under present experimental conditions. Therefore, we need to revise the experimental conditions to reduce non-specific scratching. Furthermore, as DNFB application itself caused scratching behavior, it may become another model for investigating scratching.

Histamine has been considered to be the most important substance for causing itch in humans[18] and, in most cases, histamine H_1-receptor antagonists are effective for the treatment of pruritus.[19] In the present study, however, three histamine H_1-receptor antagonists (HSR-609,[11] cetirizine[12,13] and terfenadine[14]) did not significantly affect scratching in mice caused by either DNFB application or 48/80 injection, although cyproheptadine clearly inhibited scratching. These results suggest that histamine antagonism may not be effective in inhibiting scratching in mice and that serotonin may participate in the induction of scratching. In contrast, the edematous reaction caused by the application of DNFB in sensitized mice was potently inhibited by all these agents, indicating that histamine and serotonin play an important role in inducing edema.[9] In sensitized mice, mast cells are activated following challenge with DNFB to release various mediators, including histamine and serotonin,[20] and the vasoactive amines cause an edematous reaction. Therefore, histamine plays an important role in DNFB-induced cutaneous edema, whereas it does not seem to have a central role in causing scratching. However, serotonin may play important roles in the development of both edema and scratching. We need to undertake further experiments using selective serotonin antagonists to elucidate the role of serotonin in DNFB-induced scratching. Furthermore, scratching itself may cause mediator release from mast cells as a physical stimulus and may potentiate the edematous reaction. In preliminary experiments, however, application of 25 μL of a 0.15% DNFB solution caused a high incidence of scratching without causing any edematous response in non-sensitized mice, suggesting that the scratching may not affect the edematous reaction under present experimental conditions. We also need to carefully evaluate the effect of scratching on the edematous response in the mouse ear.

Prednisolone, a potent anti-inflammatory drug, significantly inhibited DNFB-induced scratching and edema, whereas it did not affect 48/80-induced scratching. Prednisolone inhibits the activation of various inflammatory cells and the subsequent production and release of inflammatory mediators.[21,22] The application of DNFB to sensitized mice is considered to cause the activation of various cells in the skin, including sensitized mast cells, and results in the induction of edema and scratching. Prednisolone may inhibit the induction of edema and scratching by suppressing the activation of the cells involved in their development.[10] In contrast, 48/80 may cause scratching more directly than in the case of DNFB.

Dibucaine inhibited the scratching caused by both DNFB and 48/80, probably by blocking the itch sensation pathway, although this pathway is not yet established. Further experiments should be undertaken to demonstrate the existence of afferent nerve systems for sensitive itching. In contrast, opioids exhibit an analgesic action but enhance the itch sensation.[23,24] There are some reports that indicate the effectiveness of opiate antagonists for the treatment of itching in hepatic diseases.[25,26] Therefore, endogenous opioids may participate in the generation of the itch sensation and naloxone may act as an antagonist against endogenous opioids. Nevertheless, the mechanism of action of naloxone is still obscure; naloxone apparently inhibited both DNFB- and 48/80-induced scratching, suggesting that scratching behavior in mice induced by both DNFB and 48/80 share a common induction pathway.

The present results indicate that scratching behavior could be induced in mice in association with an IgE-mediated allergic cutaneous reaction and that the reaction is pharmacologically similar, but not identical, to that caused by 48/80. Although histamine is considered to participate in the formation of ear edema, it does not seem to play an important role in the generation of scratching. This animal model may serve as a model for pharmacological studies on allergic itch and as a model for research on antipruritus agents.

Acknowledgment

We are grateful to Hokuriku Seiyaku Co. Ltd (Fukui, Japan) for providing cetirizine and HSR-609.

References

1 Denman ST. A review of pruritus. *J. Am. Acad. Dermatol.* 1986; **14**: 375–92.
2 Bernhard JD. Clinical aspects of pruritus. In: Jeffers JD, Scott E, White J (eds). *Dermatology in General Medicine*, 3rd edn. New York: McGraw-Hill, 1987; 78–90.
3 Greaves MW. Pruritus. In: Champion RH, Burton JL, Ebling FJG (eds). *Textbook of Dermatology*, 5th edn, vol. 1. Oxford: Blackwell Science, 1992; 527–35.
4 Wahlgren C-F. Pathophysiology of itching in urticaria and atopic dermatitis. *Allergy* 1992; **47**: 65–75.
5 Kuraishi Y, Nagasawa T, Hayashi K, Satoh M. Scratching behavior induced by pruritogenic but not algesinogenic agents in mice. *Eur. J. Pharmacol.* 1995; **275**: 229–33.
6 Ray MC, Tharp MD, Sullivan TJ, Tigelaar RE. Contact hypersensitivity reactions to dinitrofluorobenzene mediated by monoclonal IgE anti-DNP antibodies. *J. Immunol.* 1983; **131**: 1096–102.
7 Katayama I, Tanei R, Yokozeki H, Nishioka K, Dohi Y. Induction of eczematous skin reaction in experimentally induced hyperplastic skin of Balb/c mice by monoclonal anti-DNP IgE antibody: Possible implications for skin lesion formation in atopic dermatitis. *Int. Arch. Allergy Appl. Immunol.* 1990; **93**: 148–54.
8 Sakurai T, Inagaki N, Nagai H. The effect of anti-tumor necrosis factor (TNF)-α monoclonal antibody on allergic cutaneous late phase reaction in mice. *Life Sci.* 1994; **54**: PL291–5.
9 Nagai H, Sakurai T, Inagaki N, Mori H. An immunopharmacological study of the biphasic skin reaction in mice. *Biol. Pharm. Bull.* 1995; **18**: 239–45.
10 Inagaki N, Tsunematsu M, Sakurai T, Matsuo A, Nagai H. The effect of prednisolone on IgE-dependent biphasic cutaneous reaction in BALB/c mice. *Gen. Pharmacol.* 1997; **28**: 93–7.
11 Musoh K, Ohashi T, Iwanaga Y *et al.* Non-sedating anti-allergic agent, HSR-609, inhibits allergic airway and peritoneal eosinophilia in mice. *Proceedings of the Second Asian Pacific Congress of Allergology and Clinical Immunology*, 1995; 210 (Abstract).
12 De Vos Ch, Maleux MR, Baltes E, Gobert J. Inhibition of histamine skin reaction in four animal species by UCB PO71 (cetirizine 2HCl). *Ann. Allergy* 1985; **55**: 392 (Abstract).
13 Rihoux JP, De Vos Ch, Baltes E, de Lannoy J. Pharmacoclinical investigation of cetirizine, a new potent and well-tolerated anti H1. *Ann. Allergy* 1985; **55**: 392 (Abstract).
14 Cheng H, Woodward J. Antihistaminic effect of terfenadine: A new piperidine-type antihistamine. *Drug Rev. Res.* 1982; **2**: 181–96.
15 Fjellner B, Hägermark Ö. Studies on pruritogenic and histamine-releasing effects of some putative peptide neurotransmitters. *Acta Derm. Venereol.* 1981; **61**: 245–50.
16 Hägermark Ö, Hökfelt T, Pernow B. Flare and itch induced by substance P in human skin. *J. Invest. Dermatol.* 1978; **71**: 233–5.
17 Armstrong D, Dry RML, Keele CA, Markham JW. Observations on chemical excitants of cutaneous pain in man. *J. Physiol.* 1953; **120**: 326–51.
18 Keele CA, Armstrong D. *Substance Producing Pain and Itch*. Baltimore: Williams and Wilkins, 1964.
19 Davis MG, Greaves MW. The current status of histamine receptors in human skin: Therapeutic implications. *Br. J. Dermatol.* 1981; **104**: 601–6.
20 Harvima RJ, Schwartz LB. Mast cell-derived mediators. In: Foreman JC (ed.). *Immunopharmacology of mast cells and basophils*. London: Academic Press, 1993; 115–38.
21 Church MK. Drugs in asthma treatment. In: Holgate ST, Church MK (eds). *Allergy*. London: Gower Medical Publishing, 1993; 15.1–12.
22 Toogood JH, White FA. Corticosteroids: Mechanisms of action and treatment of allergic diseases. In: Townley RG, Agrawal DK (eds). *Immunopharmacology of Allergic Diseases*. New York: Marcel Dekker, 1996; 675–706.
23 Fjellner B. Experimental and clinical pruritus. *Acta Derm. Venereol.* 1981; **61**: 1–34.
24 McMahon SB, Koltzenburg M. Itching for an explanation. *Trends Neurosci.* 1992; **15**: 497–501.
25 Bernstein JE, Swift RM. Relief of intractable pruritus antagonized by naloxone. *Arch. Dermatol.* 1979; **115**: 1366–7.
26 Jones EA, Bergasa NV. The pruritus of cholestasis: From bile acid to opiate agonists. *Hepatology* 1990; **11**: 884–7.